

WEDNESDAY AM

## 675.13

**\*LESION-INDUCED INCREASES IN LAMININ AND FIBRONECTIN EXPRESSION IN ORGANOTYPIC HIPPOCAMPAL SLICE CULTURE.** L.E. Paulman\*, M.G. Welsh, and L.S. Jones. Dept. of Developmental Biology and Anatomy, Univ. South Carolina, Sch. of Med., Columbia, SC 29209

In order to examine the role of extracellular matrix (ECM) in the mammalian CNS's response to trauma, we have used the organotypic hippocampal slice culture system (OTS) where we have already characterized the postnatal expression patterns of laminin (LN) and fibronectin (FN). OTS cultures were prepared from 4d postnatal rats, and were allowed to adapt to culture conditions for 7d prior to being manually lesioned by razor chips. The cultures were then fixed at 24h intervals for 15d post-lesion and labeled by immunofluorescent techniques with antibodies to LN and FN. Results indicated that there was an early increase in LN expression in cells and processes immediately adjacent to and on both sides of the lesion site, which peaks at 24-36h post-lesion. The LN immunoreactivity was also localized in neovasculation, which developed in the region of the lesion, paralleling the lesion course. This vascular expression was not present until 48-72h post-lesion, the time at which the non-vascular expression immediately adjacent to the lesion was decreasing. FN expression was not seen in any cells immediately adjacent to the lesion site at any of the time points studied; however, at 36-48h post-lesion, there were FN-immunopositive cells in the neuropil of the OTS near the course of the lesion. These FN-positive cells all possessed FN-positive processes that were extending toward the lesion site. This concurs with other reports showing up-regulation of FN in the CNS following physical injury, and is to our knowledge the first report of LN increase in CNS tissue following injury. These results indicate that the OTS system is a useful model with which to study the effects of physical injury in the CNS in addition to its demonstrated value in examining ischemia, anoxia and cytotoxic events. This work was supported by NS27903

## 675.15

**CNTF IS AN ENDOGENOUS AXON REGENERATION FACTOR FOR MAMMALIAN RETINAL GANGLION CELLS.** S.A. Jo\*, E.Y. Wang and L.I. Benowitz. Dept. Neurosurgery, Children's Hosp. and Harvard Med. School, Boston, MA 02115.

Neurons of the mature CNS in humans and other higher vertebrates are generally unable to regenerate damaged axons after injury. To identify factors that might promote axonal regeneration, we utilized purified rat retinal ganglion cell (RGC) cultures prepared by immunopanning (Barres et al., *Neuron* 1: 791, 1988), and tested the ability of several known neurotrophic factors in stimulating outgrowth. We found that ciliary neurotrophic factor (CNTF) and its closely related homolog, leukemia inhibitory factor (LIF) have striking outgrowth-promoting activity. The effects of CNTF and LIF were dose-dependent, with an EC<sub>50</sub> of 1-2 ng/ml. Brain-derived neurotrophic factor and several other factors which, like CNTF, contribute to cell survival, had little or no effect on axonal outgrowth from isolated RGCs. This result indicates that there is a dissociation between cell survival and outgrowth. A high molecular weight fraction from media conditioned by rat optic and sciatic nerves mimicked the effects of CNTF on retinal ganglion cells. CNTF was detected in these conditioned media by western blotting, and an anti-CNTF antibody neutralized the activity in the media. Hence, CNTF appears to be the primary polypeptide growth factor for RGCs in the optic nerve. Stimulation of axonal outgrowth from RGCs was accompanied by increased expression of GAP-43, paralleling the increased expression of this protein that occurs during regeneration *in vivo*. We conclude that CNTF is secreted from the glial cells of the optic nerve and can potentially help stimulate axonal regeneration from RGCs provided other conditions are favorable. Support: NIH EY 05960 and Boston Life Sciences, Inc.

## 675.17

**EXPRESSION OF THE ACTIN MONOMER BINDING PROTEIN BETA-THYMOGIN IS CORRELATED WITH NEURONAL GROWTH IN ZEBRAFISH.**

Roth-L.W.A., Bormann-P., Bonnet-A., Reinhard-E.\* Department of Pharmacology, Biozentrum, University of Basel, 4056 Basel, Switzerland

$\beta$ -thymosin, a 45  $\alpha$  long polypeptide which binds actin monomers and thereby prevents actin polymerisation, was found to be induced in regenerating zebrafish retinal ganglion cells and in glial cells of the regenerating nerve. During development,  $\beta$ -thymosin mRNA is transiently expressed in a specific subset of neural cells, primarily in the brain. Its staining pattern in brain often delineates present and future axonal pathways such as the tracts to the posterior commissure and the postoptic commissure, including the postoptic commissure itself. Expression of  $\beta$ -thymosin is also found in mesodermal tissue of the pectoral fins, preceding innervation by motoneurons. During development, increased  $\beta$ -thymosin levels are paralleled by increasing levels of actin. In contrast to the transient expression of  $\beta$ -thymosin, actin is constitutively expressed in the adult brain and retina, indicating that more actin is present in monomeric form during neuronal growth than in the fully differentiated nervous system. Regulation of the  $\beta$ -thymosin gene during development and regeneration is different from the regulation of two other actin filament severing genes, gelsolin and profilin, whose expression cannot be correlated with neuronal growth. This indicates that distinct mechanisms underlie the regulation of the actin cytoskeleton in different cell types and that  $\beta$ -thymosin may be a major participant in the rapid assembly and disassembly of cytoskeletal elements during neuronal growth. Future experiments aim to elucidate the function of  $\beta$ -thymosin during neuronal development and regeneration.

Supported by a grant from SNF 31-45925.95.

## 675.14

**ANTIBODY TO NERVE GROWTH FACTOR INHIBITS SPROUTING OF UNMYELINATED AFFERENT FIBERS IN THE DORSAL HORN AFTER SPINAL CORD TRANSECTION.** N.R. Krenz\*, S.O. Meakin and L.C. Weaver. The John P. Robarts Research Institute and Neuroscience Program, University of Western Ontario, London, Ontario, Canada N6A 5K8.

Spinal cord transection (SCT) results in sprouting of unmyelinated primary afferent fibers in the deep laminae of the dorsal horn. This sprouting may be responsible for the hyper-reflexive sympathetic regulation of arterial pressure, muscle spasticity and behavioral hyperalgesia that develop after cord injury. We tested the ability of an antibody to nerve growth factor (anti-NGF) to block afferent sprouting after cord injury. Unmyelinated afferent fibers respond to the presence of NGF by extending neurites, and elevated levels of NGF have been found in the cord after injury. Anti-NGF was delivered for a period of fourteen days immediately following SCT at the fourth thoracic segment. Alzet mini-pumps containing the antibody were implanted into the subcutaneous space adjacent to the seventh and eighth ribs. The neutralizing potency of the antibody to NGF was assessed *in vitro* using PC12 cells before administration to the rats. Retained potency of the antibody after fourteen day containment in the implanted pumps was confirmed by analyzing the small volume of antibody remaining in the pumps at the end of the experiment. Immunohistochemistry for calcitonin gene-related peptide (CGRP) was used to illuminate the terminal arbors of unmyelinated and lightly myelinated afferent fibers in the dorsal horn. When compared to untreated cord-transsected rats fourteen days after injury, the area of CGRP-IR fibers in anti-NGF treated rats was decreased in thoracic segments immediately below the transection (T6-9), corresponding to the dermatome of antibody administration. The area of the afferent arbor in other thoracolumbar segments rostral and caudal to the cord transection remained similar in the two groups of rats. By inhibiting afferent fiber sprouting after SCT, antibodies to NGF may also suppress the development of exacerbated reflexes in the injured spinal cord. Supported by MRC Canada.

## 675.16

**MOLECULAR CLONING OF A GENE FOR A PUTATIVE INHIBITOR TO CNS REGENERATION**

Chen, M.S.; Spillmann, A.A.\*; Huber, A.; Van der Haar, M.; M.F. Schwab. Brain Research Institute, University of Zurich, CH-8029 Zurich, Switzerland.

Mammalian neurons of CNS (central nervous system) origin regenerate poorly, if at all, after injuries. Although there is recent evidence that the intrinsic properties of a neuron affect its ability to regenerate, it is clear that the CNS environment plays an important role in determining whether a CNS neuron will regenerate or not after injuries.

Previously, a monoclonal antibody, IN-1, has been described to enhance regeneration of lesioned nerve fibers *in vivo*. Subsequently, we have characterized the myelin protein bNI-250 as an IN-2 neutralizable 250kd bovine protein which inhibits neurite outgrowth from NGF treated PC12 and DRG cells. Six peptide sequences were derived from bNI-250, and novel cDNA clones were obtained based on these peptide sequences. These cDNA clones are designated as EST (rat), Oli18 (rat), and CWP1-3 (bovine). Preliminary molecular analyses of these clones indicate that they are alternative products of a novel bovine (or its rat homologue) gene. Results from Northern blotting indicate the existence of three different transcripts from this gene. These clones thus represent either the short transcripts (~2 and 3kb in length) of the gene, or incomplete clones of the longest transcript (~5kb). In situ hybridization results demonstrate that these transcripts are expressed in oligodendrocytes as well as specific subpopulations of neurons. In agreement with bNI-250 being an integral membrane protein, hydrophobicity analysis of the predicted products of these clones suggests the existence of two hydrophobic regions. Taken together, one or more transcripts of this gene may encode an oligodendrocyte-derived neurite outgrowth inhibitor.

## 675.18

**EXPRESSION OF REGENERATION-ASSOCIATED-GENES AFTER ACUTE THORACIC SPINAL CORD INJURY AND IMMUNOLOGICAL MYELIN SUPPRESSION IN THE RAT.** G.W. Hieber\*, J.K. Dyer, J.D. Steeves & W. Tezlaif. CORD (Collaboration On Repair Discoveries) Depts. of Zoology, Anatomy & Surgery, UBC, Vancouver, Canada, V6T 1Z4.

We are currently investigating whether removal of the myelin sheath following thoracic spinal cord injury stimulates an increase in expression of regeneration associated genes (RAGs). Previous data using *in situ* hybridization with <sup>35</sup>S labelled oligonucleotide probes has shown that after a cervical spinal cord injury, without any demyelinating treatment, there is increased expression of RAGs, such as GAP-43, TAI-1, tubulin and actin. Increased RAG expression correlated with successful regeneration of rubrospinal neurons into the growth permissive environment of a peripheral nerve graft. In contrast, after a thoracic (T10) spinal cord injury there is little or no increase in RAG expression within the cell body and correspondingly a failure of rubrospinal neurons to regenerate into a peripheral nerve graft.

Recent studies have indicated that myelin is inhibitory to CNS regeneration after injury. We have previously used an immunological protocol involving intraspinal infusion of serum complement proteins and myelin-specific antibodies (anti-galactocerebroside) to partially demyelinate or transiently alter the structure of spinal cord myelin in order to remove the inhibitory influences. Application of this protocol after thoracic spinal cord injury facilitates some regeneration of brainstem-spinal neurons in embryonic chick, adult chicken, and adult rodent.

We hypothesize that there should be an increase in RAG expression in rubrospinal neurons when our demyelinating protocol is applied following a thoracic spinal cord injury. This would support the concept that the immunological removal of myelin inhibitors may not only create a permissive environment but also enhances the regenerative propensity of injured neurons.

Supported by the Canadian Neuroscience Network, MRC of Canada, SCRF (PVA) and the Rick Hansen Man in Motion Foundation.